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LAHIVE & COCKFIELD
28 STATE STREET
BOSTON, MA 02109

EXAMINER	
SCHLAPKOHL, WALTER	

ART UNIT	PAPER NUMBER
1636	

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Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/789,186	MURPHY, KENAN C.	
	Examiner	Art Unit	
	Walter Schlapkohl	1636	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *22 November 2005*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-43* is/are pending in the application.

4a) Of the above claim(s) *27-43* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-26* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

Claims 1-43 are pending in the instant application.

Election/Restrictions

Receipt is acknowledged of the papers filed 11/22/2005 in which Group I (claims 1-26) was elected.

Claims 27-43 are withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to a nonelected invention, there being no allowable generic or linking claim. Election was made **without** traverse in the reply filed on 11/22/2005.

The restriction is deemed proper and is therefore made FINAL.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 1-2, 4-5, & 7-14, and therefore dependent claims 3, 6, & 15-26, are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and

distinctly claim the subject matter which applicant regards as the invention.

Claims 1, 4, 7-9, 11 & 13-14 recite nucleotide sequences encoding either a "bacteriophage recombinase function" or a "bacteriophage anti-recombinase function" (claims 1 and 9), a "bacteriophage λ Red recombinase function" (claims 4, 7, 11 and 13) or a "bacteriophage λ anti-RecBCD function" (claims 4 and 11) or "λ anti-RecBCD function" (claims 8 and 14). Claims 1, 4, 7-9, 11 & 13-14 are vague and indefinite in that it is not clear what is meant by a recombinase "function." Does a nucleotide sequence encoding a recombinase function include proteins which are not recombinases but share some other recombinase-associated function(s)? What structures or characteristics are imparted by the recombinase or anti-recombinase *function* encoded by the nucleotide sequences?

Claim 1 recites "[a]n isolated nucleotide molecule comprising: (a) nucleotide sequences encoding a bacteriophage recombinase function; (b) nucleotide sequences encoding a bacteriophage anti-recombinase function; (c) *Ptac* promoter sequences operably linked to the nucleotide sequences of (a) and (b); and (d) nucleotide sequences encoding LacI operably linked to its native promoter. Claim 1 is vague and indefinite in that it is unclear how many sequences are encompassed by the claim.

For example, how many sequences can encode a bacteriophage recombinase function and how many are encompassed in the claimed isolated nucleic acid molecule? Does Applicant intend that one nucleotide sequence (perhaps consisting of many subsequences) encodes for a single recombinase or does Applicant intend multiple sequences in concatamers that all encode for a single recombinase function? Similarly, how many nucleotide sequences are capable of encoding LacI operably linked to its native promoter? Does Applicant intend that more than one copy of LacI operably linked to its promoter is present in the isolated nucleic acid?

Similarly, claims 2, 4-5 and 7-14 all refer to an isolated nucleic acid molecule comprising "sequences" in the plural that encode either a single function or a single gene (LacI or λ *gam*, *exo* or *bet*, or an origin or replication which confers low copy number on a vector) in such a manner as to be unclear with regard to how many sequences are encompassed.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 1, 4 and 7-8 are rejected under 35 U.S.C. 103(a) as being unpatentable over Stewart et al (U.S. Patent No. 6,355,412) in view of Poteete et al (*Journal of Bacteriology* **182**(8):2336-2340, 2000).

Note: For purposes of this rejection claim 1 has been interpreted to encompass an isolated nucleic acid molecule that encodes at least one recombinase (e.g., λ *exo* or *bet*) and one "anti-recombinase" (e.g. λ *gam*), each under the control of a *Ptac* promoter, and further comprising a lacI gene under the control of its native promoter.

Stewart et al teach an isolated nucleic acid comprising a the recombinase λ Red α (*exo*) and Redβ (*bet*) genes which can placed under the control of the *Ptac* promoter (see entire document, especially column 25, lines 45-67 and column 26, lines 19-22). Stewart et al teach that the *Ptac* promoter is tenfold stronger than lacUV5 (column 26, lines 19-22). Stewart et al also teach the use of a lacI gene when sequences encoding such polypeptides as RecE/T or λ *exo* or *bet* are transcribed via lac operon regulatory sequences (column 25, lines 59-67). Stewart

et al teach the use of such an isolated nucleic acid in methods of cloning and to promote homologous recombination in *Escherichia coli* (see e.g., column 11, lines 3-8; column 26, lines 34-35; and columns 45-50, claims 2-11 and 15). Stewart et al teach that "the ability to control the expression of the recombinase sequences such that expression can be regulatable (e.g. inducible) and such that a wide range of expression levels can be achieved is beneficial to the performance of the methods of the invention" (column 24, lines 50-54). Stewart et al further teach an isolated nucleic acid comprising both recombinase (λ *exo* and *bet*) as well as anti-recombinase (λ Red γ (*gam*)) "functions" (see reference to pBADαβγ in column 25, lines 17-19).

Stewart et al do not explicitly teach an isolated nucleic acid comprising sequences encoding an anti-recombinase function such as λ Red γ (*gam*) under the control of a *Ptac* promoter.

Poteete et al teach an isolated nucleic acid comprising the Red genes (*gam*, *bet* and *exo*) of phage λ under the control of a *Ptac* promoter for use in a method of homologous recombination within *E. coli* K12 (see entire document, especially page 2337, Table 1, Strains TP507 and TP522-TP645, except for TP605; page 2338, left column, second full paragraph). Poteete et al teach that higher frequency of recombination is seen with *E. coli*

strains in which the recBCD gene cluster is replaced by all three Red genes of phage λ: *bet*, *exo* and *gam* (page 2336, first full paragraph). Poteete et al also teach that, in addition to proceeding at high efficiency, "Red-mediated recombination between a short linear DNA molecule and a circular homologue may represent a simpler recombination pathway than any of the previously characterized pathways for conjugational or transductional recombination" and that "these properties of efficiency and (relative) simplicity recommend the hybrid phage-bacterial recombination system for research on general recombination mechanisms" (page 2336, right column, second full paragraph).

It would have been obvious for one of ordinary skill in the art to replace the phage λ *exo* and *bet* genes operably linked to the *Ptac* promoter of Stewart et al with the phage λ *exo*, *bet* and *gam* genes of Poteete et al because Poteete et al teach that higher frequency of recombination is seen with *E. coli* strains in which the recBCD gene cluster is replaced by all three Red genes of phage λ and Stewart et al teach the use of isolated nucleic acids comprising λ *exo* and *bet* in methods of cloning and to promote homologous recombination in *E. coli*.

One of ordinary skill in the art would have been motivated to combine the isolated nucleic acids of Stewart et al and

Poteete et al because Poteete et al teach that phage λ Red (*bet*, *exo* and *gam*) mediated recombination "may represent a simpler recombination pathway than any of the previously characterized pathways for conjugational or transductional recombination" and that "these properties of efficiency and (relative) simplicity recommend the hybrid phage-bacterial recombination system for research on general recombination mechanisms" and Stewart et al teach the use of their nucleic acid in methods of cloning and homologous recombination. Furthermore, one or ordinary skill in the art would be motivated to use the *Ptac* promoter as taught by both Stewart et al and Poteete et al, especially as noted by Stewart et al, in situations where high levels of expression for the λ Red genes are necessary.

Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent evidence to the contrary, there would have been a reasonable expectation of success to result when combining the isolated nucleic acid comprising phage λ *bet*, *exo* and *gam* genes under the control of the *Ptac* promoter as taught by Poteete et al in the nucleic acid of Stewart et al comprising λ *bet* and *exo* sequences as well as the lacI gene under the control of its native promoter.

Claims 1-26 are rejected under 35 U.S.C. 103(a) as being unpatentable over Stewart et al in view of Poteete et al as applied to claims 1, 4 and 7-8 above, and further in view of Datsenko et al (*PNAS* **97**(12):6640-6645, 2000).

To reiterate briefly, Stewart et al in view of Poteete et al teach a nucleic acid comprising nucleic acid sequences comprising a recombinase function (λ *bet* and *exo*), an anti-recombinase function (λ *gam*), both under the control of the *Ptac* promoter, as well as a lacI gene operably linked to its native promoter. Stewart et al in view of Poteete et al further teach such a nucleic acid in a recombinant bacterial *E. coli* K12 host. Stewart et al further teach that the homologous recombination of the invention can be performed in any host in which heterologous expression of the λ *Red* genes is possible (Stewart et al at column 28, lines 11-15). Stewart et al further teach that in a preferred embodiment, the sequences of homology used for recombination may be derived from pathogenic bacteria important in infection (claim 20) including but not limited to (entero)pathogenic *E. coli* (claims 20-21), *Pseudomonas aeruginosa* (claims 23-24) and *Mycobacterium tuberculosis* (claims 25-26) (see Stewart et al at column 32, lines 15-36; column 26, lines30-35).

Stewart et al in view of Poteete et al do not teach such a nucleic acid further comprising an origin of replication sequence which confers low copy number on a vector comprising such a nucleic acid. Nor do Stewart et al in view of Poteete et al teach such a nucleic acid wherein the origin of replication is temperature sensitive.

Datsenko et al teach the use of an isolated nucleic acid comprising λ *bet*, *exo* and *gam* sequences and further comprising a temperature sensitive origin or replication in a procedure that 1) has allowed for over 40 different disruptions in the *E. coli* K12 chromosome without failure, and 2) should be widely useful, especially in genome analysis of *E. coli* and other bacteria (see entire document, especially the reference to pKD20 in the paragraph bridging pages 6641-2; page 6640, right column, 2nd full paragraph; and page 6640, last sentence of the Abstract). Datsenko et al further teach that preliminary studies in which attempts were made to make chromosomal mutations by using the multicopy Red plasmid pTP223 and PCR products with short homology extensions were unsuccessful (ibid). Datsenko et al then decided to make low copy plasmids such as pKD20 which expresses *gam*, *bet* and *exo*, has an optimized ribosome-binding site for efficient translation of *gam*, and which has the further advantage that its temperature-sensitive replicon allows for its

easy elimination at 37 deg. C (ibid). This improvement was made, Datsenko et al teach, because multicopy plasmids might interfere with recombination by acting as competitive inhibitors (page 6644, first full paragraph). Datsenko et al explicitly teach such a nucleic acid in an *E. coli* K12 recombinant host, but note that their method should be "widely useful" and easily extended to use in other bacteria (page 6645, last paragraph).

It would have been obvious for one of ordinary skill in the art to use the temperature sensitive origin or replication of Datsenko et al with the nucleic acid of Stewart et al and Poteete et al because all three references teach efficient recombination in *E. coli* with the use of nucleic acids comprising λ *Red* sequences. It would have been obvious for one of ordinary skill in the art to extend the use of such nucleic acids to hosts such as (entero)pathogenic *E. coli*, *Mycobacterium tuberculosis*, and *Pseudomonas aeruginosa* because the use of DNA derived from such species is explicitly taught in Stewart et al, and both Stewart et al and Datsenko et al teach that the host cell can be any host cell in which the λ *Red* genes can be expressed.

One of ordinary skill in the art would have been motivated to combine the temperature sensitive origin or replication of Datesenko et al with the nucleic acid of Stewart et al and

Poteete et al because Datsenko et al teach that such an addition allows for easy elimination of the vector, which if present in multiple copies, might result in inhibition of recombination. Furthermore, the temperature sensitive nature of the origin or replication specifically allows for its easy curing by growth at 37 deg. C. One of ordinary skill in the art would also have been motivated to extend the host range beyond *E. coli* K12 to pathogenic *E. coli*, *M. tuberculosis* and *P. aeruginosa* for use in genome analysis (as taught by Datsenko et al) of bacteria involved in infection (as taught by Stewart et al).

Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent evidence to the contrary, there would have been a reasonable expectation of success to result when utilizing the isolated nucleic acid comprising phage λ *bet, exo* and *gam* genes under the control of the *Ptac* promoter as taught by Poteete et al and Stewart et al with the temperature sensitive origin or replication as taught by Datsenko et al in hosts including pathogenic *E. coli*, *P. aeruginosa* and *M. bacterium*.

Conclusion

No claims are allowed.

Certain papers related to this application may be submitted to the Art Unit 1636 by facsimile transmission. The faxing of such papers must conform with the notices published in the Official Gazette, 1156 OG 61 (November 16, 1993) and 1157 OG 94 (December 28, 1993) (see 37 C.F.R. § 1.6(d)). The official fax telephone number for the Group is (571) 273-8300. Note: If Applicant *does* submit a paper by fax, the original signed copy should be retained by Applicant or Applicant's representative. NO DUPLICATE COPIES SHOULD BE SUBMITTED so as to avoid the processing of duplicate papers in the Office.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to (571) 272-0547.

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Any inquiry concerning rejections or objections in this communication or earlier communications from the examiner should be directed to Walter A. Schlapkohl whose telephone number is (571) 272-4439. The examiner can normally be reached on Monday through Friday from 8:30 AM to 5:00 PM. A phone message left at this number will be responded to as soon as possible (i.e., shortly after the examiner returns to his office.)

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Dr. Remy Yucel can be reached at (571) 272-0781.

Walter A. Schlapkohl, Ph.D.
Patent Examiner
Art Unit 1636

January 24, 2006

NANCY VOGEL
PRIMARY EXAMINER